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Middle and late holocene micromammal pathologies from Cueva Tixi (Tandilia Range, Buenos Aires Province, Argentina)

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ABSTRACT

Paleopathology in bones of very small mammals has rarely been studied. Different types of osseous lesions of mammals weighing under 0.2 kg, recovered from the Holocene strata of Cueva Tixi archaeological and paleontological site (Tandilia range, province of Buenos Aires, Argentina), are described and discussed in this report. Several types of trauma, entheseal changes, degenerative joint diseases, and probable osteomyelitis were identified. The lesions were chronic, indicating that the animals were able to survive a period of convalescence, although in many cases the decreased capacity for locomotion likely was significant. These pathological findings open research avenues for very small mammals that usually are not considered in archaeological disease studies.

1. Introduction

Faunal paleopathology has become increasingly important in recent decades. Earlier studies described primarily macrofauna wherein the observed pathologies derived mainly from congenital defects, injuries, bone responses to acute trauma, and repeated articular mechanical stress (Harcourt, 1971; Siegel, 1976; Harris, 1977; Baker and Brothwell, 1980; Duff, 1987; Clark, 1994; de Cupere et al., 2000; Izeta and Cortés, 2006; Acosta Hospitaleche et al., 2012; Rafuse et al., 2013). Until relatively recently, micromammal (< 1.0 kg body weight) paleopathology studies tended to be avoided because of difficulties with analysis of small size specimens and perhaps indirectly because of lack of attention to differential diagnosis.

This report is a first approach to the study of osseous disorders that affected mammalian species weighing under 0.2 kg. The study sample comes from the Holocene strata of Cueva Tixi archaeological and paleontological site (Tandilia range, province of Buenos Aires, Argentina; Mazzanti, 1997; Quintana, 2016). The observed lesions indicate long term conditions that imply post-incident survival time. The aims of this report are to describe the variability of observed pathological findings, to consider their etiologies and their consequences in terms of behavioral responses, and to elaborate possible differential diagnoses. The most important contribution of this research is related to previous absence of such analysis for micromammals recovered from archaeological or paleontological settings.

2. Materials and methods

2.1. Cueva Tixi archaeological site

Cueva Tixi is a cave located in La Vigilancia hill of the Tandilia range (province of Buenos Aires, Argentina) at 190 m above sea level, near the valley of San Pedro creek (Mazzanti, 1993) (Fig. 1). The covered area is 40 m2, with 1.80 m average height. The sedimentary sequence reveals six clearly identified strata (Martínez and Osterrieth, 2001). Its unit A is a layer of calcium carbonate from the back of the cave, without fauna or cultural remains (Mazzanti and Quintana, 2001, 2002). The remaining strata (B to F) are characterized by increasing grain size to the base, from clayey silt to coarse sandy silts. The faunal remains are distributed in four levels (B to E). The oldest level is separated into E lower (late Pleistocene) and E upper (Middle Holocene), considering their chronology and their archaeological and faunal content (Mazzanti, 1993; Quintana, 2001a).

Cueva Tixi has a chronological time span from the Late Pleistocene (10,375 ± 90 14C years BP) to the final Late Holocene, determined by six radiocarbon dates on charcoal samples from indigenous hearths (Mazzanti, 1993, 1997) processed by the NSF Arizona AMS Facility laboratory at the University of Arizona USA. The top of the sequence (stratum B) was dated in 170 ± 80 14C years BP. This result is at the limit of dating method reliability, but the entire archaeological and paleontological context indicates pre-conquest times (Fig. 2).

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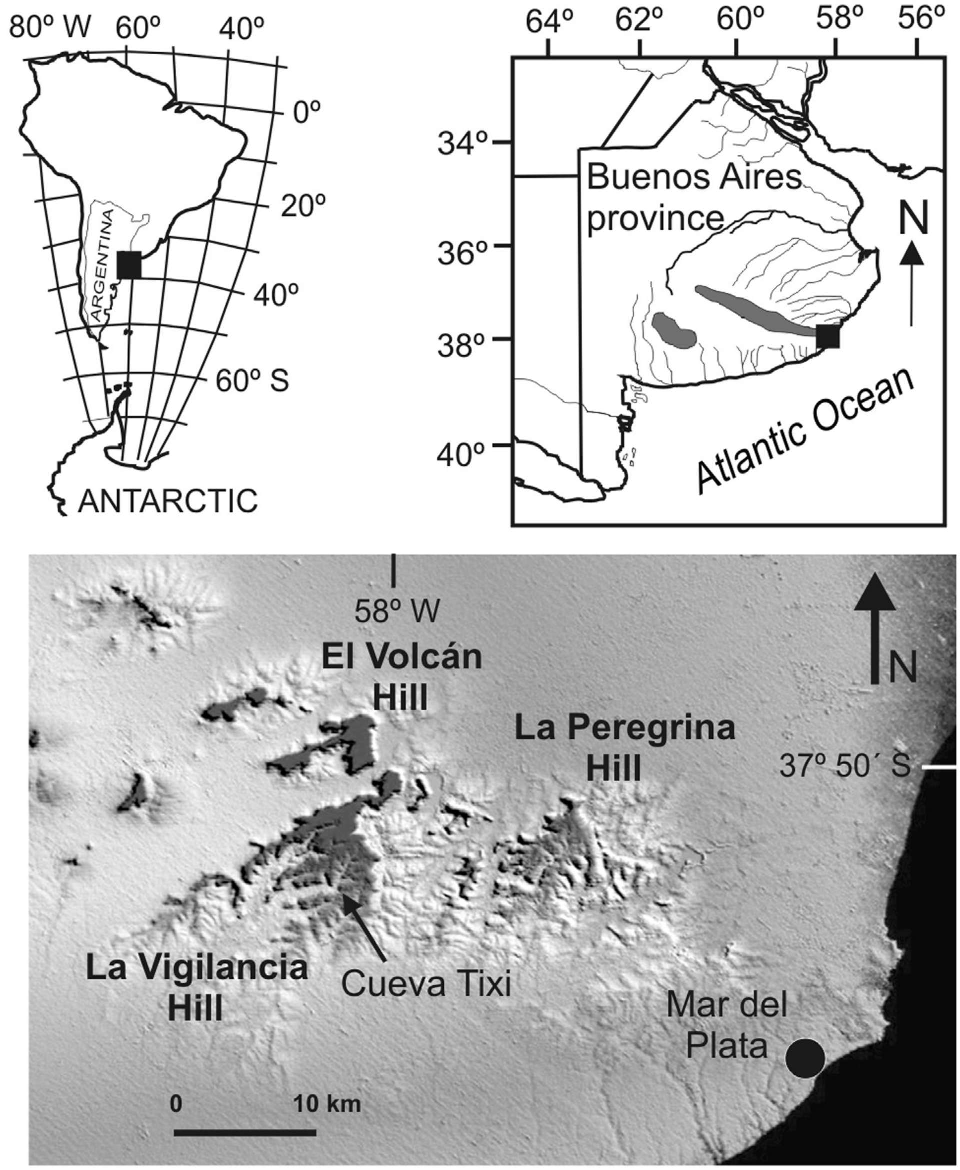

Fig. 1. Location map for Cueva Tixi.

The sedimentary deposits contain four archaeological levels (1–4) of hunter-gatherer societies (Mazzanti, 1993, 1997). This cave has a large fossil record, from which analyses have established (a) the paleontological sequence and human survival strategies from the Late Pleistocene to the Late Holocene in Eastern Tandilia (Quintana, 2001a), and (b) the recovered archaeological collection (lithics, faunal remains, pottery, hearths, bone technology) (Mazzanti, 1993, 1997; Martínez et al., 2013). The methodological aspects of the excavation, data survey techniques, and the description of the human subsistence strategies inferred from Cueva Tixi, are presented in Mazzanti (1993, 1997) and Mazzanti and Quintana (2001, 2002).

2.2. Cueva Tixi microvertebrates

Among the small vertebrates (weighing less than 5.0 kg), microvertebrates are characterized by mass smaller than 1.0 kg. This small size conditions methodologies of sampling, collection, and analysis, because it presents distinctive variables at all degrees of taphonomic alteration (Korth, 1979; Andrews, 1990; Stahl, 1996; Szabolcs and Virág, 2009). Further, it is important that studies of micromammals, among microvertebrates, have led to development of new analysis methodologies (Andrews, 1990; Fernandez-Jalvo and Andrews, 1992; Stahl, 1996).

The micromammals analyzed in this report belong to the scientific collection of the Laboratory of Archeology (LARQ) of the National University of Mar del Plata (Buenos Aires province, Argentina). The entire microvertebrate collection includes 72,116 skeletal remains that belong to different taxa: didelphid marsupials, rodents, bats, frogs, birds, snakes, and fishes (Quintana, 2016). Micromammals are represented by 18 species that average less than 0.2 kg weight; snakes are represented by four species, and fishes by two. Species of birds and frogs continue to be identified (Table 1). Among mammals, rodents are the most abundant (NISP: 66,789), followed by didelphid marsupials (NISP: 339).

Several zoological sources for the assemblage were identified in this large sample (Quintana and Mazzanti, 2001; Quintana, 2004, 2016),

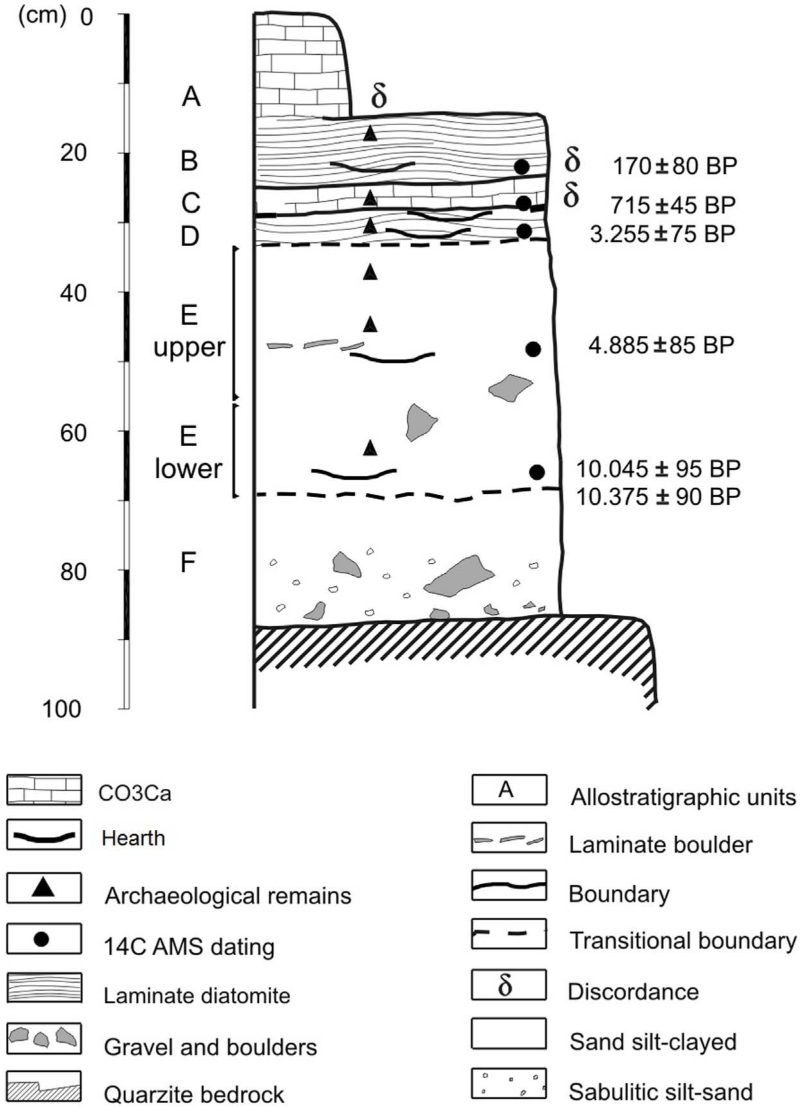

Fig. 2. Stratigraphy and chronology of Cueva Tixi.

including ophidians (snakes) that died naturally during periods of dormancy, and caviid rodents and fishes that were hunted by indigenous societies during the last millennium (Quintana, 2001b, 2004, 2016). The majority of the microvertebrates, including cricetid rodents, didelphids, birds, and frogs (identified along the entire sequence), and caviid rodents recovered only from the lower levels (Late Pleistocene to Middle and initial Late Holocene), were the prey of nocturnal raptors (*Tyto alba*) (Quintana, 2016).

2.3. Methods

Element identification was conducted considering comparative collections housed in LARQ, and faunal atlases (France, 2009). All the specimens mentioned above were observed escopically and with a portable magnifying glass, in order to identify any pathological evidence. Given that basic gross pathological macro-manifestations are the same or very similar in bones across different species, lesions were described and photographed, and differential diagnosis was considered in each instance, within the obvious limits of isolated bone analysis. Human paleopathology manuals and textbooks were consulted for comparisons (Rogers and Waldron, 1995; Aufderheide and Rodríguez Martín, 1998; Ortner, 2003; Pinhasi and Mays, 2008; Rothschild and Martin, 2006; Waldron, 2009; Grauer, 2012).

3. Results

Overt pathologies were identified in bones from all the strata, excepting the lower E. We have categorized them as fractures, entheseal changes, osteoarthroses, and other pathologies. After meticulous macroscopic inspection of the entire sample, it was observed that only 17 bones were affected, the remainder exhibiting no overt pathology.

3.1. Fractures

Two specimens show different traumatic consequences of excessive externally-applied mechanical force of probably-blunt nature (Bennike, 2008; Judd and Redfern, 2012). The first specimen is a healed complete transverse fracture is seen in the distal diaphysis of a left femur from an adult caviomorph rodent (*Ctenomys talarum*) (LARQ 761, stratum D, initial Late Holocene). Some taphonomic bone loss is present, but large

Table 1
Microvertebrate record from Cueva Tixi.

Scientific name		Common name
MAMMALS	Didelphidae marsupials	
	Lutreolina crassicaudata	Little water oposum
	Lestodelphys halli	Patagonian oposum
	Monodelphis dimidiata	Southern short-tailed opossum
	Monodelphis sp. nov.	Fat-tailed mouse opossum
		Sigmodontinae rodents
	Akodon azarae	Azara's grass mouse
	Bibimys torresi	Torres's pink-nosed mouse
	Calomys sp.	Drylands vesper mouse
	Holochilus brasiliensis	Web-footed marsh rat
	Necromys sp	Bolo mouse
	Oligoryzomys flavescens	Yellow pygmy rice rat
	Oxymycterus rufus	Red hocicudo
	Pseudoryzomys simplex	Brazilian false rice rat
	Reithrodon auritus	Bunny rat
	Scapteromys cf. *S. aquaticus*	Argentine swamp ratCaviomorph rodents
	Ctenomys talarum	Tuco-tuco
	Cavia aperea	Guinea pig, cuiz
	Galea tixiensis	Small Guinea pig Chiroptera
	Genera and species indet	Bat
AVES	*Agelaioides badius*	Bay-winged cowbird
	Anthus lutescens	Yellowish pipit
	Cisthotorus platensis	Grass wren
	Nothura maculosa	Spotted nothura
	Thinocorus rumicivorus	Least seedsnipe
	Sicalis luteola	Grassland yellow-finch
	Sturnella loyca	Long-tailed meadowlark
	Genera and species indet	–
SERPENTES	*Clelia rustica*	Musurana
	Bothrops alternatus	Yayará
	Philodryas patagoniensis	Green racer snake
ANURA	Genera and species indet.	Frogs
TELEOSTEI	*Rhamdia* cf. *R. quelen*	Catfish
	Corydoras cf. *C. paleatus*	Pepper cory

callus formation, notable angulation, and slight periostic reaction are seen clearly and without evidence of infection. The alterations observed on the femoral head are taphonomic; some of the articular surface is missing and the feature is altered by root action (Fig. 3).

The second specimen is the distal end of the diaphysis of the right tibia of an adult cricetid rodent (LARQ 766, stratum E upper, Middle Holocene). It shows a complete and fully healed transverse fracture that includes a large callus, angulation, displacement, and overlapping of the broken ends (Fig. 4). An important implication is that these animals survived long enough post-injury for complete healing to occur. In both cases, the observed lesions would have produced significant functional limitation of the affected limbs.

A likely case of spiral fracture was also identified in an adult left femur of an undetermined cricetid rodent (LARQ 760, stratum C, final Late Holocene). The diaphysis is thickened and deformed by a large callus with neoformation of pointed osseous areas and overlapping proximal and distal segments. The pointed osseous foci may have resulted from healing of associated muscle damage or from small fracture fragments that healed back to the adjacent bone. It is not clear whether a secondary infection occurred. The limb would have been notably shortened following healing, being thus limiting but not necessarily debilitating (Fig. 5a and b).

3.2. Enthesial changes

Three specimens show osseous alterations in areas of muscle and tendon insertion (entheses), consequences of chronic excessive force vectors relating to muscle and tendon attachments and movements. These depressions and growths have differential diagnoses that include chronic microtrauma, external trauma, muscle tear or avulsion, periosteal damage, inflammation, or healing of a lesion by fibrosis and/or mineralization.

Studies of entheses are useful to understand stress points (Henderson

Fig. 3. Traumatic lesion with angulation in a left femur of *Ctenomys talarum* (LARQ 761, initial Late Holocene). (a) cranial view; (b) lateral view; (c) caudal view; (d) medial view, scale = 10 mm. Note: The cranial area of the diaphysis shows radicular erosion.

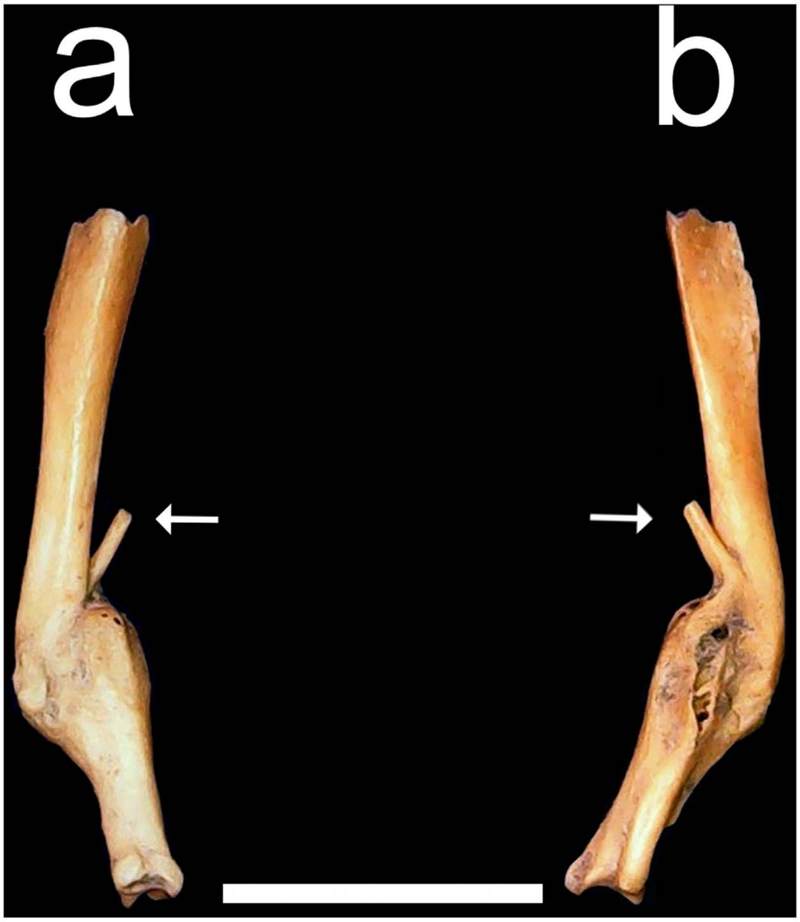

Fig. 4. (a) caudal view; (b) cranial view. Healed transverse fracture with a large callus, angulation, displacement, and overlapping of the broken ends of a right tibia of a cricetid rodent (LARQ 766, Middle Holocene), scale = 5 mm. Note: arrows indicate the location of a fibula fragment.

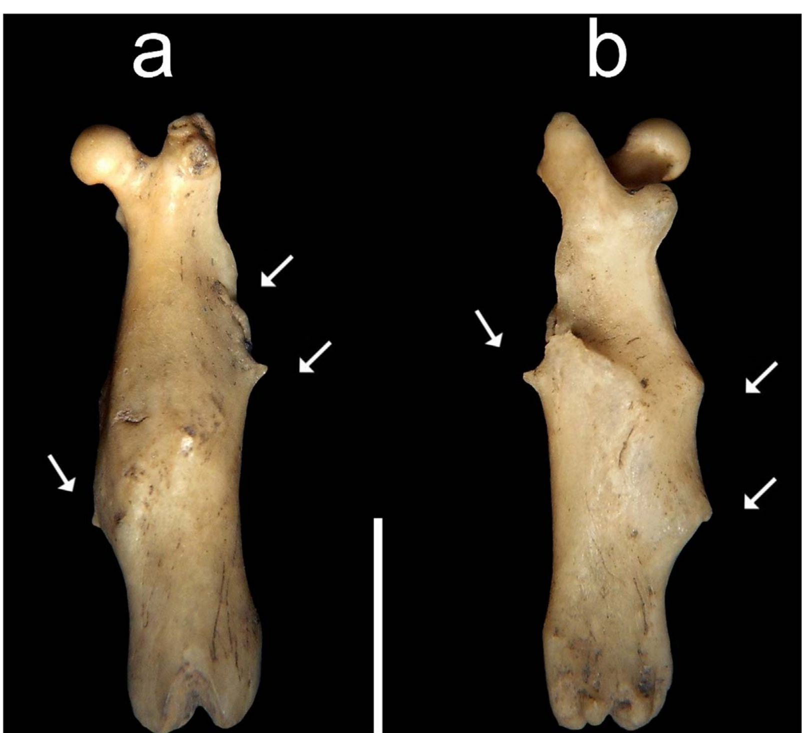

Fig. 5. (a) caudal view; (b) cranial view. Adult left femur of a cricetid rodent (LARQ 760, Late Holocene), with a spiral healed fracture, scale = 5 mmcules.

Fig. 6. Enthesial changes in proximal portions of left femora of cricetid rodents. (a) a cranial view (LARQ 755, Late Holocene), scale = 5 mm; (b) a lateral view (LARQ 765, Middle Holocene), scale = 5 mm; (c) caudal area (LARQ 763, Late Holocene), scale = 5 mm. Note: arrows point to the osseous proliferation.

et al., 2013, 2016; Villote and Knusel, 2013). For example, a longitudinal bone outgrowth is observed in the proximal epiphysis of a left femur of the cricetid rodent *Reithrodon auritus* (LARQ 755, stratum B, final Late Holocene), between the greater trochanter and the neck (Fig. 6a). It is located in the fibrocartilaginous insertion zone of the *obturator externus*. This muscle inserts on the inner area of the obturator foramen, on the pubic and ischial rami, and on the fossa of the greater trochanter. It supports the lateral rotation of the thigh (Stone and Stone, 1990; Cantarella, 1999).

A left adult femur of a cricetid rodent of *Necromys* sp. (LARQ 765, stratum E upper, Middle Holocene) shows a large bone growth on the third trochanter (Fig. 6b). This is the area of insertion of the *gluteus maximus* muscle, which originates at the inferior and superior rami of the pubis and inserts on the upper portion of the *linea aspera*. These muscles adduct the thigh and support lateral rotation and flexion (Stone and Stone, 1990; Cantarella, 1999).

Finally, an isolated spicule of bone proliferation is seen in the mid-diaphyseal caudal area of a left femur of *R. auritus* (LARQ 763, stratum D, initial Late Holocene), oriented towards the proximal end of the bone (Fig. 6c), in the zone of insertion of the *adductor brevis* muscle. The *adductor minimus* muscle also inserts in this area (Stone and Stone, 1990; Cantarella, 1999).

3.3. Osteoarthroses

Several specimens show signs of degenerative joint disease (Ortner, 2003; Helmtrud and Tilley, 2007; Pinhasi and Mays, 2008; Waldron, 2012). The distal epiphyses of two left humeri, from a *Ctenomys talarum* (LARQ 762, stratum D, initial Late Holocene) and a *R. auritus* (LARQ 768, stratum E upper, Middle Holocene) (Fig. 7a and b) have osteophytes at the distal margin of the articulation. Articular bone erosion is visible (Fig. 7b), with a small porous depression and probable post-necrotic subchondral bone. The latter lesion may have been caused by fragmentation or destruction of the overlying articular cartilage that began in sub-articular bone (osteochondrosis), or osteoarthritis- initiating loss of articular cartilage. Both pathological conditions involve the same areas of the articulation and show similar alterations, although the underlying etiology is different. Osteochondrosis can have genetic implications in many animal species (Olsson, 1982; Resnick et al., 1995).

The lesion seen in a distal epiphysis of an adult right femur of an undetermined cricetid rodent (Fig. 7c; LARQ 759, stratum C, final Late Holocene) is associated with significant erosion and probable necrosis. The differential diagnosis includes articular trauma, infection, epiphyseal fracture, loss of vascular supply during development, or a penetrating wound possibly caused by a predator. Acute trauma and infection seem to be the most likely possibilities, considering the bone reaction observed.

The distal articulation of an adult left ulna of an undetermined cricetid rodent (LARQ 767, stratum E upper, Middle Holocene) also demonstrates multiple proliferative osteoarthritis, probably secondary to a completely healed fracture with slight misalignment (Figs. 7d and 7e). An area of osseous neoformation with central cavitation also is seen in the distal third of the diaphysis. Differential diagnosis of the latter pathology is difficult and would include fracture-associated pseudoarthrosis, avulsion, penetrating wound, abscess or hematoma, or blunt trauma.

Finally, another distal epiphysis of adult left femur of *R. auritus* (LARQ 771, left, stratum E upper, Middle Holocene; Fig. 7f), shows massive periarticular proliferative alterations, without porotic intraarticular erosion, and with some taphonomic alteration.

3.4. Other pathologies

Two adjacent metacarpals of an undetermined adult rodent (LARQ 769, stratum E upper, Middle Holocene) show massive deformation of the proximal articulation, with both proliferative and erosive alterations (Fig. 8a and b). An irregular excrescence in the proximal third of the diaphysis is seen in one of the specimens. All these pathologies probably were associated with trauma (Cushner and Morwessel, 1992; Ortner, 2003) that produced osteomyelitis secondary to introduced infection from a penetrating wound. The differential diagnosis could include *myositis ossificans* (calcification of muscular tissues) or osteitis

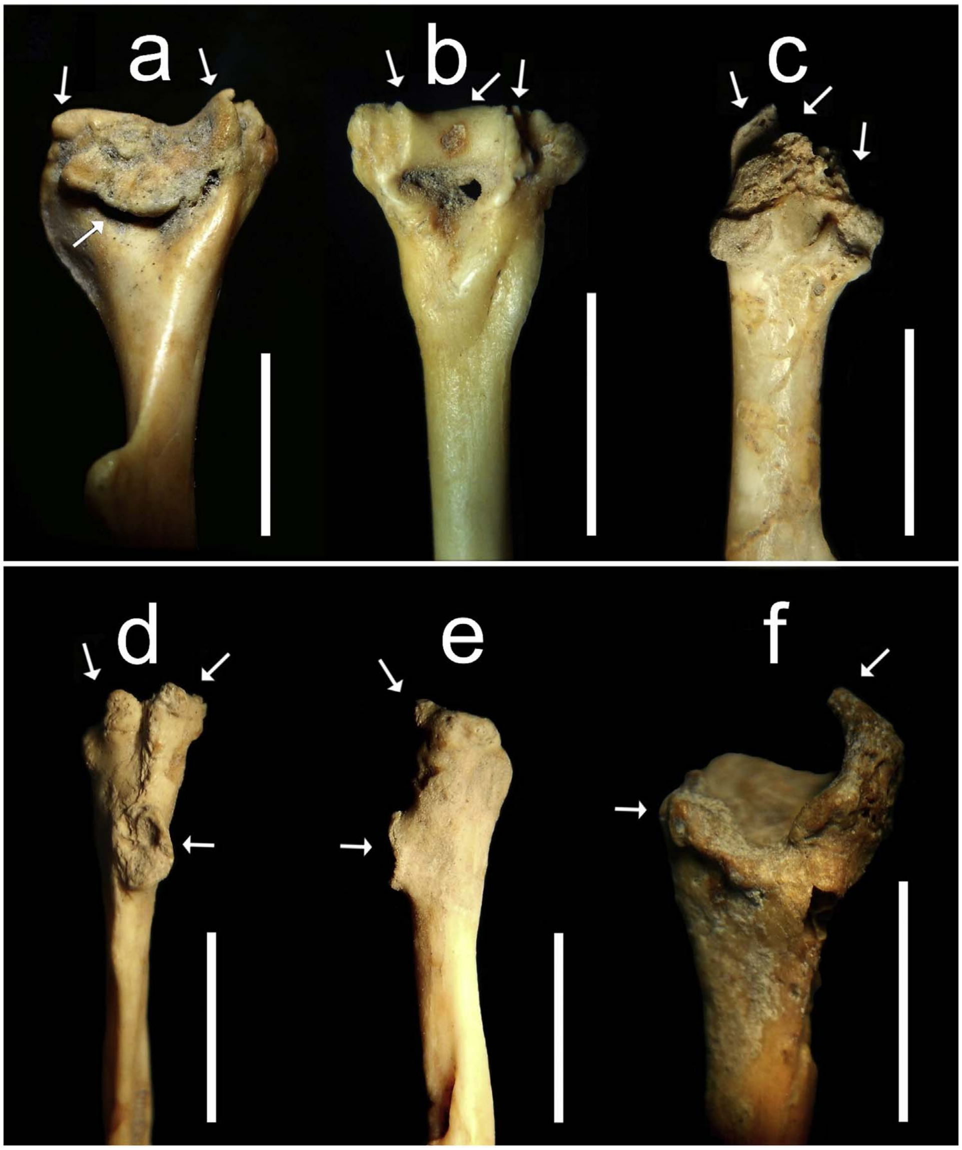

Fig. 7. Osteoarthrosis. (a) humeral distal epiphysis of *C. talarum* (LARQ 762, Late Holocene), scale = 5 mm; (b) humeral distal epiphysis of *Reithrodon auritus*, caudal view (LARQ 768, Middle Holocene), scale = 5 mm; (c) femoral distal epiphysis of a cricetid rodent (LARQ 759, Late Holocene) with significant erosion and necrosis, cranial view; (d) cranial view and (e) lateral view, of the distal articulation of a left ulna of a cricetid rodent (LARQ 767, Middle Holocene), with proliferative osteoarthrosic alterations, osseous neoformation, and cavitation, scale = 5 mm; (f) distal epiphysis of adult left femur of *R. auritus* (LARQ 771, Middle Holocene) with periarticular alterations, caudal view, scale = 5 mm. Note: arrows point to the most important features for each bone.

(Ortner, 2003). The osseous excrescence could be related to a benign neoplasm, but this interpretation is less plausible.

Traumatic *myositis ossificans* is seen in the middle third of a left tibial diaphysis of a cricetid rodent (LARQ 764, stratum E upper, Middle Holocene) in the area of fusion with the fibula (Fig. 8c). A periostic reaction, with both irregular bony neoformation and numerous osteolithic structures, is present in the area of the lesion.

The lesion observed in the caudal view of the proximal third of a left adult femur of *R. auritus* (LARQ 770, stratum E upper, Middle Holocene) (Fig. 8d) encompasses a wider area and is more complex than the last case. A differential diagnosis of the irregularity and thickening of the trochanter and diaphysis includes osteomyelitis, *myositis ossificans*, infectious or inflammatory periosteal diseases, and vascular or lymphatic disorders.

Finally, two other lesions are more difficult to diagnose. Alteration of the bone structure is observed in the proximal end of an adult left femur of *R. auritus* (LARQ 756, stratum B, Late Holocene), mainly in the neck and the trochanter area, with deformity and an augmented central cavitation (Fig. 9a and b). The proximal third of the diaphysis also is affected. The feature probably was caused by blunt or penetrating local bone trauma, although no fracture traces are seen. Severe muscle injury or avulsion, and bone avulsion with or without a primary fracture of the greater trochanter, are possible explanations for this lesion.

On the other hand, an adult right femur of a cricetid rodent (LARQ 758, stratum C, final Late Holocene) shows massive deformity of the proximal end, with absence of the head and the trochanters (Fig. 9c and d). Probable differential diagnoses are hip joint dislocation, nonunion fracture, or ischemic femoral head necrosis. In chronic dislocations, a pelvic pseudoarthrosis almost always is seen. A femoral neck fracture may occur in some instances, leaving the femoral head in the acetabulum (Nunamaker, 1985; Witsberger et al., 2007). In any case, locomotion would have been reduced severely.

4. Discussion

Micromammal paleopathology has received little attention in

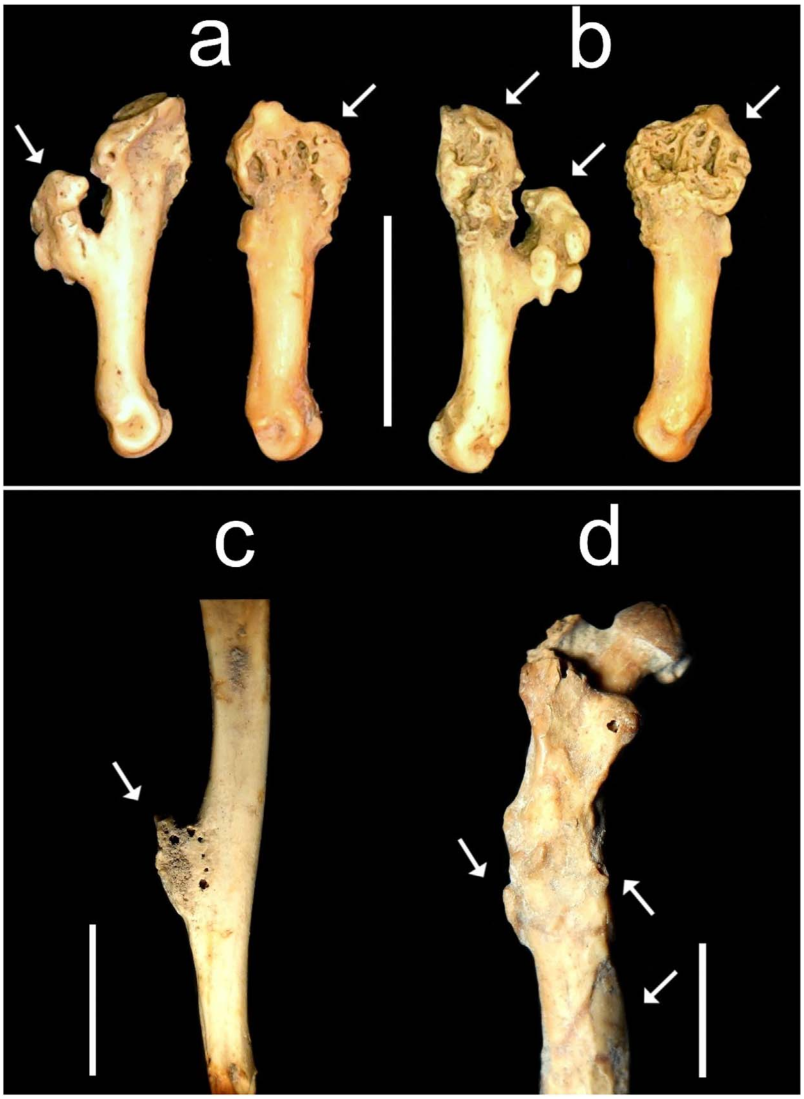

Fig. 8. Other pathologies. (a) medial view; (b) lateral view. Metacarpals of an undetermined rodent (LARQ 769, Middle Holocene) with proliferative and erosive articular alterations that suggest a differential diagnosis including *myositis ossificans*, osteitis, osteomyelitis or (more distantly) neoplasm, scale = 5 mm; (c) middle third of a left tibial diaphysis of a cricetid rodent (LARQ 764, Middle Holocene) with *myositis ossificans* and a periostic reaction, lateral view, scale = 5 mm; (d) proximal third of a left adult femur of *R. auritus* (LARQ 770, Middle Holocene), with cortical irregularity and thickening, caudal view, scale = 5 mm. Note: arrows point to the most important features for each bone.

osteological research, although the information that this discipline may provide is very useful for understanding the ethology and stress-derived responses of low-weight mammals. The lesions identified in this report mainly are due to trauma (Figs. 3–5) and severe stress on musculoskeletal tissues (Figs. 6–9), in some cases associated with secondary pathologies.

In the first case, healed fractures with angulation and overlapping ends occurred (Figs. 3 and 4), causing shortening of the bone with resulting abnormal gait or non-use of the limb. These lesions would have limited the normal movement of the affected limbs. Osseous alterations, probably caused by heavy blunt trauma, are also seen (Figs. 7c, d, e, 8c, 9a, b). In fact, the probable osteomyelitis cases reported (Fig. 8a, b, d) also may be consequences of accidents such as a fracture (although it seems not to be the cases in Fig. 8 because no callus was identified) or a penetrating wound (prick with a thorn).

All the pathologies are chronic; the animals that suffered them lived at least several weeks after the events. In consequence, they were able to perform behaviors that allowed them to obtain food and protect themselves from predators, even though several of these injuries would have produced important locomotor limitations. In these cases, the most plausible defense from predators likely was to remain temporarily quiet and hidden.

In completely healed fractures, some immobilization is necessary for development of a normal callus, so it surprising that the animals could obtain food during that period, considering also the effects of pain. It is possible that small mammals with a single injury in one limb may remain sufficiently mobile to survive if food and water is available nearby. In the case of immature animals, a reasonable functional recovery of some locomotion may involve periods of only 3–4 weeks (Dennis Lawler, personal communication; Herron, 1981).

Previous taphonomic studies of Tixi Cave microvertebrate accumulations established that the cause of death of all these specimens most probably was depredation by the barn owl (*Tyto alba*) (Quintana, 2016), so reduced locomotion must have resulted in a lower escape potential. All the analyzed bones correspond to rodents that the barn owl is known to consume, while some bone disorders of *R. auritus* may relate to its great abundance in the fossil record of Cueva Tixi (Quintana, 2016).

Regrettably, complete skeletons were not recovered, but this is expected when avian predators predominate in the paleontological record. This fact precludes more specific characterizations, although this first approximation of small mammal paleopathology opens new perspectives and generates problem-oriented questions for the study of osteological samples that usually are not considered for disease studies.

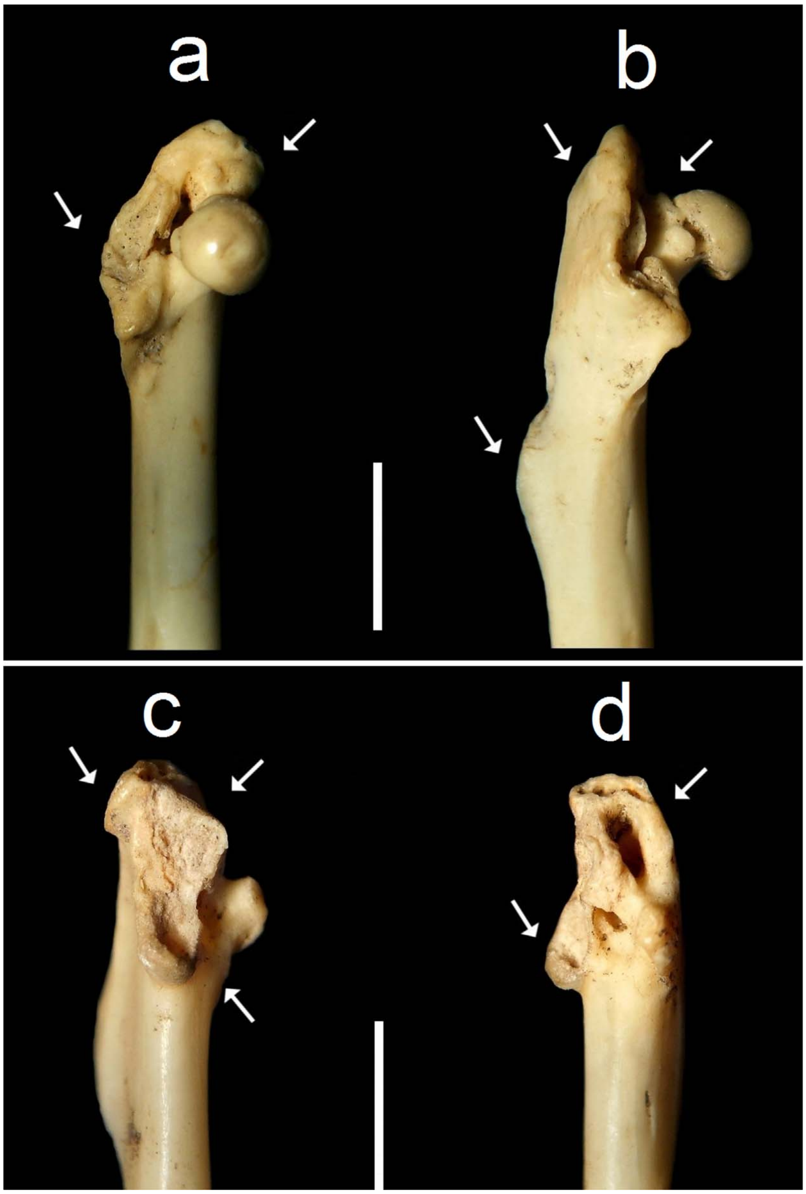

Fig. 9. (a) cranial view; (b) medial view. Proximal end of *R. auritus* (LARQ 756, Last Holocene) femur with alteration of the bone structure, probably caused by local trauma, scale = 5 mm; (c) cranial view; (d) lateral view of the right femur of a cricetid rodent (LARQ 758, Last Holocene) with massive deformity of the proximal end scale = 5 mm. Note: arrows point to the most important features for each bone.

Finally, this type of research has a great potential to support new areas of inquiry, such as the history of identified diseases, circumstances of death, and survival capacity. This work also demonstrates the increasing the use of differential diagnoses in paleopathology.

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